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Identification of the Organisms Causing Soft-Rot Disease in Vegetables in the Fields of the Tsuruoka District

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Summary

In order to identify the organisms causing the soft-rot of vegetables in the fields of Tsuruoka, a total of 264 soft rot bacterial strains were isolated from 15 kinds of diseased plants in 1984. Out of them, 52 strains were tested for identification. All the strains produced grayish-white colonies on NA medium and yellow ones on MD medium. They were Gram-negative, nonspore-froming, facultatively anaerobic, peritrichously flagellated rods, and fermented glucose. They were catalase-positive, oxidase-negative and induced soft rot in various fresh vegetables. On the basis of their pathogenicities and 66 bacteriological characteristics, 36 strains were identified as *Erwinia carotovora* subsp. *carotovora*. The remaining 16 strains produced reducing substances from sucrose and produced acid from maltose, palatinose, and α-methyl glucoside. Consequently they seemed to belong to *Erwinia carotovora* subsp. *atroseptica*.

Introduction

A wide variety of vegetables has been cultivated in the Tsuruoka district. Many vegetables suffer seriously from various diseases each year. Among the diseases, bacterial soft-rot caused by members of the carotovora group of the genus *Erwinia*[10,13] is known to inflict damage on chinese cabbage, cabbage, radishes, potatoes, and many other vegetables.

The members of the carotovora group are divided into 4 species and 2 subspecies[10,13]. However, up to the present study, little information has been available on the species and subspesies responsible for soft-rot disease in the fields of Tsuruoka.

The present study was carried out to identify the soft-rot organisms. These were isolated from diseased vegetables.

A part of this work was presented at the Annual Meeting of Tohoku Division of Phytopathological Society of Japan in October 1985.

Materials and Methods

Isolation of soft rot bacterial strains. Soft rotted tissues of various plants were collected in the fields of Hie, Komakibara, Minden, and Takasaka around Tsuruoka district in 1984. Small pieces of the tissues from margins of the rotted areas were suspended in 10 ml of sterilized water. One ml of a suitable diluent of the suspension was poured into petri dishes with modified Drigalski's medium (MD), which was the selective medium for the soft rot bacteria[18]. After incubation for 48 hrs. at 25°C, *Erwinia*-like colonies having the yellow nucleus and transparent margins on the plates were selected and purified by isolating single colonies from repeated plating. The strains isolated were maintained in nutrient agar slants(NA) at room temperature. The stock cultures were plated on NA plates and incubated for 48 hrs. at 25℃ before they were tested for identification. All tests were performed at 25℃ unless otherwise stated.

Bacteriological characteristics. The strains

were tested for the 66 bacteriological chracteristics as described by Cowan and Steel[1], Dye[3,4], Goto and Takikawa[6], Graham[7], Lelliott[10], and Schaad[13].

Pathogenicity tests. The pathogenicites of the bacterial strains were tested on slices of storage tissues of various plants. An aqueous suspension of cells at the density of $5\sim6\times10^7$ CFU/ml for each strain was used as an inoculum. After surface sterilization of slices of carrot, potato tuber, radish, and petioles of chinese cabbage in 70% ethanol for 3 min, the slices were inoculated according to Shih-Tien and Kuo-Ching Tzeng[14]. The inoculated slices were maintained in moistend petri dishes at 25℃. Observations of the rotting of the slices were made 24~72 hrs. after inoculation.

Results and Discussion

In the present study, a serious degree of soft rot disease was found to occur in many kinds of vegetables from the fields of the Tsuruoka district. A total of 264 soft rot bacterial strains were obtained from 15 kinds of diseased plants. Out of the total strains, 52 strains were used for identification, as shown in Table 1.

While *Bacillus subtilis*, *B. polymyxa*, *B. megatherium*, *Pseudomonas marginalis*, and pectolytic strains of *Pseudomonas*, *Clostridium*, spp., and *Flavobacterium* spp. were proved to be involved in the decay of living plant tissues[12], bacteria of the genus *Erwinia* were the most prevalent[12].

It was discovered that in Japan there were differenecs in the growth rates in Uschinsky's and Cohn's solutions, gas production from carbon compounds, and optimum growth temperature among the 46 bactetial strains from various soft rotted vegetables.[15]. Moreover, most of the soft rot bacteria isolated from soils and diseased plants in the northern part of Japan was found to belong to *Eriwinia aroideae*(=*E. carotovora*)[18].

The 52 strains tested in the present study formed graysh white-colonies on NA medium and yellow ones on MD medium. They were Gram-negative, non-

Table 1. Origin and species of the soft rot bacterial strains

Source plant	No. of strains isolated	No. of strains tested for identification	No. of strains identified as *Erwinia carotovora* subsp.	
			carotovora	*atroseptica*
A Cabbage	27 (9)	4		4
B Carrot	25 (6)	4	2	2
C Cauliflower	13 (5)	2	2	
D Chinese cabbage	99(18)	8	4	4
E Chinese chive	12 (4)	6	4	2
F Chinese mustard	7 (3)	2	2	
G Leaf mustard	5 (2)	4	4	
H Lettuce	10 (3)	4	4	
I Mioga	8 (3)	2	2	
J Onion	7 (2)	2	2	
K Potato	6 (2)	4	2	2
L Radish	21 (7)	4	2	2
M Spinach	9 (2)	2	2	
N Taro	9 (6)	2	2	
O Turnip	6 (2)	2	2	
	264(72)	52	36	16

( ) : No. of plants used for the isolation of bacteria

Table 2. Bacteriological characteristics of bacterial strains isolated from soft rotted tissues of various plants

Characteristics	Isolated strains																
	A[a] (4)[b]	B (4)	C (2)	D (8)	E (6)	F (2)	G (4)	H (4)	I (2)	J (2)	K (4)	L (4)	M (2)	N (2)	O (2)	E. cc[i]	E. at[j]
Growth in broth	+[c]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flagellation	P[d]	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Gram stain	−[e]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
O-F test	F[f]	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Indole production	−	−	−	−	−	−	−	−	−	+	−	−	+	−	−	+	−
H_2S production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Blue pigment	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Red. sub. from sucrose	+	+	−	+	v[g]	−	−	−	−	−	v	v	−	−	−	−	+
Gluconate oxidation	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pectate degradation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrolysis of arbutin	−	v	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
casein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
starch	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Levan formation	+	v	−	v	v	−	−	−	−	+	v	−	−	−	−	−	−
Limus milk	rac[h]	rac	rac	rac	rac	rac	rac	rac	rac	rac	rac	rac	rac	rac	rac	rac	rac
Acetoin production	+	+	+	+	v	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red test	+	−	+	v	v	−	v	+	+	+	+	+	+	+	+	+	+
Gas from glucose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Phenylalanine deaminase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Decarboxylase																	
Arginine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lysine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glutamic acid	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lecitinase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Phosphatase	−	l	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Urease	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Growth in 5% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 37℃	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
rhamnose	+	+	+	+	+	+	v	+	+	+	+	v	−	+	+	+	+

Characteristics	A[a] (4)[b]	B (4)	C (2)	D (8)	E (6)	F (2)	G (4)	H (4)	I (2)	J (2)	K (4)	L (4)	M (2)	N (2)	O (2)	E. cc[i]	E. at[j]
fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
maltose	+	v	−	v	v	v	−	−	−	−	v	v	−	−	−	−	+
sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
raffinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
palatinose	+	v	−	v	v	−	−	−	−	−	v	v	−	−	−	−	+
inulin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
dextrin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
soluble starch	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
salicin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
sorbitol	v	v	−	v	v	−	−	−	−	−	v	v	−	−	−	−	−
inositol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
dulcitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
aesculin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
α-methyl glucoside	+	v	−	v	−	−	−	−	−	−	v	v	−	−	−	−	+
Utilization of formate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
malonate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
lactate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
tartrate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
galacturonate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pathogenicity on carrot	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
potato	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
radish	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
chinese cabbage	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

a) mark of plants shown in Table 1.
b) No. of bacterial strains tested for identification
c) + : positive reaction
d) P : peritrichous
e) − : negative reaction
f) F : fermentative
g) v : variable among strains
h) rac : reduced, acidified, and curled
i) E. cc : *Erwinia carotovora* subsp. *carotovora*
j) E. at : *Erwinia carotovora* subsp. *atroseptica*

sporeforming, facultatively anaerobic, peritrichously flagellated rods, and fermented glucose. They were catalase-positive, oxidase-negative, phosphatase-negative, and liquefied gelatin and pectate. When the bacterial suspensions were inoculated to the slices of storage tissues of plants, the initial symptoms appearing within 24 hrs. were typical water-soaked lesions. Within 1～2 days after the appearence of the inital symptoms, the lesions developed throughout the whole slices. From the bacteriological characteristics and pathogenicities, the present strains were considered to be members of the carotovora group of the genus *Erwinia*[10,13].

The carotovora group consists of 4 species and 2 subspecies[10,12]. Species of the genus *Erwinia* belonging to the carotovora group, namely *Erwinia carotovora* subsp. *carotovora* (*E. cc*), *E. carotovora* subsp. *atroseptica*(*E. ca*)and *E. chrysanthemi*(*E. chr*)[12], were usually refered to as soft rot bacteria.

There were several characteristics distingushing the species of the carotovora group[4,7,10,13]. On the basis of the pathogenicities and the 66 bacteriological characterisitics, including major diagnostic tests the distingushing the species in the carotovora group[10,12,13], 36 strains were demonstrated to belong to *E. cc* (Table 1, 2). The remaining 16 strains from cabbage, carrot, chinese cabbage, chinese chive, potato, and radish produced reducing substances from sucrose and produced acid from maltose, α-methyl glucoside and palatinose. Consequently they seemed to belong to *E. ca.* However, further examinations such as serological tests[16] seemed to be necessary to identify the strains accurately.

E. chr was a member of the carotovora group. The organism was known to cause bacterial stem-rot of potato[2,16,17], stalk-rot of corn[8,9] and foot-rot of rice[5]. Only *E. cc* and *E. chr* were found to be associated with the soft-rot, and *E. chr* was isolated from carrot, celery, corn, potato, rhyncostylis, and welsh onion in Taiwan[14]. On the other hand, *E. rhapondici* was reported to cause soft rot of onion in Japan[11]. However, *E. chr* and *E. rhapontict* were not found in the present study.

It may be concluded from the results that *E. cc* and possibly *E.* ca, both of the carotovora group of the genus *Erwinia*, were mainly responsible for the soft-rot in various vegetables in the Tsuruoka district.

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Literature cited

1) Cowan, S. T. (1974). Cowan and Steel's Manual for the Identification of Medical Bacteria. 2 nd ed. Cambridge Univ. Press.
2) Dickey, R. S. (1981). *Erwinia chrysanthemi*, Relation of eight plant species to strain from several hosts and to strains of other *Erwina* species. Phytopathology 71. 23-29.
3) Dye, D. W. (1968). A taxonomic study of the genus *Erwinia*. 1. The amylovola group. N. Z. J. Sci. 590-607.
4) Dye, D. W.(1969). A taxonomic study of the genus *Erwinia*. 11. The carotovora group. N. Z. J. Sci. 12. 81-97.
5) Goto, M. (1979). Bacterial foot rot of rice caused by a strain of *Erwinia chrysanthemi*. Phytopathology 69. 213-216.
6) Goto, M. and Y. Takikawa. (1984). Methods for identification of plant pathogenic bacteria (in Japanese). Shokubutsu-boeki (Plant Protection) 38. 339-340, 385-389, 432-437, 479-484.
7) Graham, D. C. (1972). Identification of soft rot bacteria. Pro. 3 rd Int. Conf. Plant. path. Bact. 273-279. Wageningen.
8) Hartman, J. R. and A. Kelman(1973). An improved method for the inoculation of corn with *Erwinia* spp. Phytopathology 63. 658-663.
9) Hoppe, P. E. and A. Kelman. (1969). Bacterial top and stalk rot disease of corn in Wisconsin. Plant Dis. Reptr. 53. 66-70.
10) Lelliott, R. A. (1974). Genus *Erwinia in* Bergey's Manual of Determinative Bacteriology, 8 th ed. The Williams and Wilkins Co., Baltimore. 332-340.

11) Ohuchi, A., T. Ohsawa, and J. Nishimura (1983). Two pathogenic bacteria *Erwinia rhapontici*(Millard 1924)Burkholder 1948 and *Pseudomonas marginalis* pv. *marginalis* (Brown 1918) Stevens 1925, causing a soft rot of onion. Ann. Phytopath. Soc. Japan 49. 619–626.

12) Perombelon, M. C. M. and A. Kelman. (1980). Ecology of the soft rot erwinias. Ann. Rev. Phytopathol., 18. 361–387.

13) Schaad, N. W. (1980). Laboratory Guide for Identification of Plant Pathogenic Bacteria. Bacteriol. Commit. Amr. Phytopath. Soc., Minesota.

14) Shih-Tien Hsu, Kuo-Ching Tzeng.(1981). Species of *Erwinia* associated with soft rot disease of plants in Taiwan. Pro. 5th Int. Conf. Plant. Path. Bact. 9–18. Cali.

15) Takimoto, S.(1951). Studies on the soft rot organisms of cultivated plants in Japan. 11. The physiological character of the causal organisms (in Japanese). Shokubutsu-Byogai-Kenkyu. 4. 178–179. Kyoto, Japan.

16) Tanii, A. (1984). Studies on the blackleg disease of potato in Hokkaido. Rep. Hokkaido Pref. Agr. Exp. St. 45. 1–104.

17) Tominaga, T. and K. Ogasawara.(1979). Bacterial stem rot of potato caused by *Erwinia chrysanthemi*. Ann. Phytopath. Soc. Japan 45. 474–477.

18) Tsuyama, H.(1962). Studies on the soft rot disease of Chinese cabbage caused by *Erwinia aroideae* (Towns.) Holland. Bull. Inst. Agr. Res. Tohoku Univ., 13. 221–345. (in Japanese with English summary).

鶴岡市周辺で分離した野菜類軟腐病細菌の同定

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摘　要

1984年，鶴岡市周辺の日枝，民田，小真木原および高坂の各地区で軟腐病に罹病した15種の野菜類から，262系統の病原菌を分離した．これらのうち，各野菜から分離した52系統について同定試験を行なった．これらの系統は，いずれもグラム陰性，通性嫌気性の同毛桿菌で運動性を示し，ゼラチン液化，カタラーゼ活性は陽性であった．さらにグルコースを発酵的に分解し，ハクサイ，ニンジンなどに病原性があり，アラビノースなどの糖類から酸を生成することなどから *Erwinia* 属の *carotovora* 群の軟腐病菌と判定された．この *carotovora* 群の種の分類基準的性質を含む66項目の細菌学的諸性質から，36系統が *Erwinia carotovora* subsp. *carotovora* と同定された．残りの16系統はスクロースから還元物質を，マルトース，パラテノースおよび α-メチルグルコシドから酸を生成し，さらに血清学的検討などが必要であるが *Erwinia carotovora* subsp. *atroseptica* と考えられた．以上の結果，鶴岡市周辺の野菜類軟腐病の発病にはこれらの2種の軟腐病菌が関与していることが示された．